AUTOMATIC METAPHASE FINDING BY INTER-CHROMOSOME EXTREMA PROFILE ANALYSIS

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Abstract - **In this paper we report our experiences with a new texture coarseness measure, as a step towards automation of metaphase finding in cell proliferation studies. This measure is highly specific to grey-level inter-chromosome coarseness features in microscopic images of metaphase spreads, and allows to quantify the texture of the cytological objects analysing the intensity profile between chromosome-extrema samples. Chromosome fragments produce patterns of pixels at low-resolution, and the local neighbourhood of their individual extrema presents a characteristic coarseness, along intensity profiles on random-oriented test lines. Results of its use on images of fields of metaphases and artefacts are compared with some representative texture measures, outperforming metaphase detection and artefacts elimination. This coarseness feature provides a specific metaphase signature that can be used in conjunction with other morphological and textural parameters for automated metaphase discrimination.**

Keywords – **metaphase finding, texture, coarseness, profile, extrema.**

I. INTRODUCTION

The microscope discrimination of metaphase spreads is a method widely and routinely used for the detection of chromosomal aberrations in medical genetics, toxicological studies of mutagenicity, cell proliferation evaluation for drugs safety, and radiation health monitoring, amongst others.

The increasing demands on health services to provide genetic counselling, pregnancy screening, evaluation of drugs in cellular proliferation and radiation health monitoring have added to the pressures on cytogenetic laboratory services. The task involved in locating a number of individual metaphases is very time-consuming, even at low resolutions. In both, routine and research environments, the involved task are tedious and tiring, but the importance and consequences of the results of screening process demand consistency and accuracy from highly trained and qualified staff. The combined effect of these factors, coupled with the omnipresent need to avoid further cost, has prompted and accelerated the design and development of systems for automated metaphase spread location.

Several approaches and systems known as *metaphase finders* have been proposed. The first instrument for semi-automated metaphase finding was described in a paper published in 1963 [1]. This system was initially concerned to locate metaphase and was based on a conventional optical microscope with a scanning stage using video imaging. Other attempts to semi-automate metaphase finding using a closed-circuit TV camera were reported in [2,3]. More recent works report the use of commercial metaphase finders and their performance [4,5,6]. Although significant progress has been made in automatic metaphase finding technology, the total time spent in the analysis of slides and a greater accuracy still represent important challenges to biomedical engineers.

In the present study, we concentrate our efforts in extracting a texture measure from the inter-chromosome profile information, achieving results that substantially enhance metaphase recognition and artefact discrimination. The texture measure proposed was tested in a current cell proliferation study. Working in a tenfold magnification and a visual field of 610x477µm, this profile feature allows the identification of variable-shaped metaphases in the presence of many artefacts. The identification performance was compared with that of classical texture features such as RED [7] and those derived from grey-level co-ocurrence matrices: contrast, entropy, energy, angular second moment [8]and MDWRE [9].

II. METAPHASE TEXTURAL INFORMATION

The human expert identifies metaphase spreads mainly by observing the internal texture produced by the chromosomes inside. In this sense, the microscope images show that texture in metaphase is composed of cytological material and image noise, but it also comprises digitalized, sub-sampled and overlapped chromosome fragments. This composition organizes grey-level variations and specific pits (chromosome fragments) into characteristic *signatures* along intensity profiles on random-oriented test lines. Our experience, and that of other researchers, has shown that relationships between extrema features are meaningful characteristics of such signatures [10]. Moreover, the semi-structural character of such textures (with the sub-sampled chromosomes constituting the *texels*) makes them suitable for run-length analysis [11], and stereological-feature extraction by profile analysis along test lines [12].

Grey-level images I(x,y) are often studied as surfaces, with intensity seen as height, and texture appearing as relief whose extrema features combine intensity and shape information. We propose to simplify 2D feature analysis by interpreting the statistical features of 1D profile samples between extrema dots. The mean and the standard deviation of intensity height along such profiles, that is, the linear *RMS-roughness* value, cannot serve alone to discriminate different signatures. In the other hand, a 2D RMS-roughness evaluation averages-out all features, while in the present study we seek inter-extrema texture signatures, which are best evaluated by analysing just 1D profiles between extrema points, and considering the

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Abstract

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ranges, that is, the largest differences between maximal and minima.

III. INTER-EXTREMA PROFILE TEXTURE FEATURE (IEPR)

In a profile or region containing N height (grey level) samples, their values can be ranked to obtain the *n-order statistics*, with n=1,..., N. Besides traditional RMS-roughness measures, statistical rank filters like the median, or the trimmed mean [10] can be used to obtain simple coarseness descriptors. In particular, the *median rank filter* is the N/2-order statistics, if N is even, and average of the (N-1)/2 and (N+1)/2 values for N odd, and it constitutes a robust RMS-roughness estimator. These observations prompted us to focus on the very distributions of ranges (the largest difference between maximal and minima in each profile), and device a simple coarseness feature. While the median rank filter (a "robust" RMS roughness estimator) is representative of height differences, coarseness is better described by the distribution of larger height differences. Thus, the *histogram median* (not the rank filter median) is used once all profile ranges (the N-order statistics) are counted. Besides extracting the median of the range population, we select only those profiles (1) between chromosome intensity minima (at each chromosome pattern), and (2) within a window representing the inter-extrema mean separation. Note that such profiles are equivalent to those of diameter samples across the neighbourhood of each minimum. Our tests in fact show no difference, provided that the neighbourhood size is the same as the window size representing the inter-extrema mean separation. We formalize in mathematical terms the above description as follows.

Let $\wp$ be the set of N points P_i (x_i, y_i), with $i=1,\dots,N$, and whose integer coordinates, x_i, y_i, lie within the analysed object boundary. These points P_i are actually the coordinates of the extrema (chromosome or artefacts dots) of an intensity-surface image $I(x,y)$, see Fig. 1b. We choose pairs (P_i , P_j), $i \neq j$ at random, within the window intervals.

To define the profile samples (Fig. 1c) along test lines, let $\wp_{ij} = \{\ell_t = (1-t)P_i + tP_j, t \in [0,1]\}$ be the straight line between the points P_i and P_j, and let $I_{ij}(\ell_t)$ be the grey level at ℓ_t. then:

$$I_{ij}(\ell_t) = \begin{cases} I(P_i) & if \quad t = 0 \\ I(P_j) & if \quad t = 1 \end{cases} \qquad (1)$$

are the endpoint intensities. Note that in a discrete space, $I(x_t, y_t)$ may not be defined. In such a case, its value is estimated by a bilinear interpolation, from the nearest neighbours. Let

$$dif_{ij} = \max_{\ell_t \in \wp_{ij}} I_{ij}(\ell_t) - \min_{\ell_t \in \wp_{ij}} I_{ij}(\ell_t) \qquad (2)$$

be the range of the profile-intensity defined by the straight-line ℓ_t, where t∈[0,1]. The coordinates of the initial point P_i and the final point P_j of the profile are within a rectangular window where $|x_i - x_j| \leq \eta \quad and \quad |y_i - y_j| \leq \eta$; in our case η=3. Since chromosome intensities vary randomly, their extrema location and intensities are random variables, and so their difference.

Let $H(dif_{ij})$ be the histogram of all ranges, defined in the equation (2), over the set $\wp$, with grey level bins in [0,255].

Let $\tilde{H}$ be the median of the histogram H, then we define

$$IEPR = \tilde{H} \Big/ (I_{max} - \min_{i,j}\{\min_{\ell_t \in \wp_{ij}} I_{ij}(\ell_t)\}) , \qquad (3)$$

where I_{max} is the maximum saturation grey level of the image and $t \in [0,1]$. Thus, *IEPR* is a texture measure of local coarseness, along the intensity profile between extrema. Normalization allows proper comparison under different contrast condition.

Denominator $I_{max} - \min_{i,j}\{\min_{\ell_t \in \wp_{ij}} I_{ij}(\ell_t)\}$ is never null, since profile endpoints include always the chromosome (or artefact) minima.
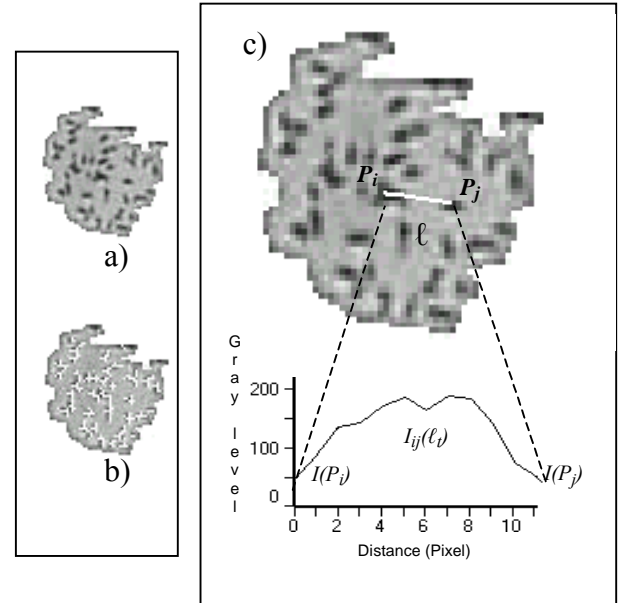

Fig. 1. Inter-chromosome (or inter-extrema) profile analysis. a) metaphase spread, b) detected extrema in a horizontal and vertical scanning of figure 1 (a), c) profile intensity graphic between two extrema points P_i and P_j.

IV. EXPERIMENTAL RESULTS

To show the performance of IEPR, as a discriminating feature in the classification of metaphases, we have applied it to the problem of metaphase finding in cell proliferation studies [13].

A. Test sets

Twenty-two slides from different experiments, and six donors where randomly chosen to create two test sets, composed of 76 metaphases and 191 artefacts.

Each test image was digitised on a 512x480-pixel grid with grey-level resolution of 256 levels.

It is important to remark that artefacts on slide image present wide variation in shape size and texture. This indeterminacy makes artefacts to have a high possibility of being a source of errors (false positives, mainly) in the automatic metaphase-finding problem. Moreover, the probability of artefacts is extremely high (one metaphase for a thousand of artefacts in mitotic index analysis). Fortunately, a large percentage of these artefacts are outside the size range of metaphases we want to score, and it is then possible to apply an outlier exclusion criterion. Nevertheless, the remaining artefacts are the most difficult to discriminate, as many of them may be similar to the objects of interest. The metaphase correctly recognized represents the true positive metaphase subset, denoted as TP.

B. Evaluation of IEPR for the test sets

IEPR was calculated for all objects from the two test sets (metaphase spreads and artefacts). Results are summarized in Table 1. The nuclei and artefacts objects miss-classified as metaphase represents the false-positives metaphase subset, denoted by FP. Comparison of performances included IEPR, MDWRE, relative extrema density (RED)[7] and the most meaningful measures from grey-level co-occurrence matrices (0° angular relationship): contrast, energy, entropy and angular second moment (ASM) [8].

TABLE 1.
IEPR PERFORMANCE COMPARISON WITH OTHER TEXTURE FEATURES.
PERFORMANCE=(TP-FP)/ TOTOBJ

	TP metaphase	FP metaphase	Performance
IEPR	96%	5%	0.9079
MDWRE	84%	14%	0.7105
Contrast	96%	25%	0.6447
RED	74%	29%	0.4473
Energy	32%	48%	0.0263
ASM	33%	65%	- 0.2895
Entropy	18%	75%	- 0.3684

V. DISCUSSION AND CONCLUSIONS

We have proposed and evaluated in this work an inter-chromosome specific measure on profile range distributions, used as a characteristic signature of metaphase spreads, as compared with present artefacts. This signature relates local range distributions with the local coarseness characteristically produced by chromosome fragments.

Based on the percentage of true positives (TP) and false positives (FP), a comparison between IEPR and other texture feature's performances is achieved. The performance of IEPR, as a texture feature, surpasses by 22% the best of the compared features (MDWRE) for metaphase finding (Table 1).

Variograms, run-lengths and co-occurrence features describe better spatial dependency and extrema separation relationships, while intensity signatures describe the kind of coarseness differences observed among stimulated nuclei, artefacts, cluttered or dispersed chromosomes. Other approaches (e.g. multi-resolution, Markov random fields and Gabor filters) require higher resolution and precious processing time to give meaningful discrimination results.

The proposed coarseness measure represents a reliable and cost effective metaphase signature that can be used in conjunction with other morphological and textural parameters for automated metaphase discrimination.

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